CLAIMS

What is claimed is:

1. A method of preparing a crude sample for detecting at least one molecular species of interest, comprising:

(a) acquiring a crude sample containing at least one molecular species of interest, one or more rough components that are larger than the molecular species of interest, and one or more fine components that are smaller than the molecular species of interest;

(b) separating from the molecular species of interest at least a portion of the rough component and, optionally, at least a portion of the fine component of the crude sample, thereby producing a cleared sample comprising the at least one molecular species of interest;

(c) optionally, at least partially denaturing the at least one molecular species of interest contained in a cleared sample; and

(d) pre-focusing the at least one molecular species of interest in the cleared sample, wherein steps (a) through (d) are performed for a number of times sufficient to bring the concentration of at least one molecular species of interest in the sample up to the level of detection.

2. The method of Claim 1 wherein step (c) further comprises removing insoluble contaminants.

3. A method of detecting at least one molecular species of interest, comprising:

(a) acquiring a crude sample containing at least one molecular species of interest, one or more rough components that are larger

than the molecular species of interest, and one or more fine components that are smaller than the molecular species of interest;

(b) separating from the molecular species of interest at least a portion of a rough component and, optionally, at least a portion of the fine component of the crude sample, thereby producing a cleared sample comprising the at least one molecular species of interest;

(c) optionally, at least partially denaturing the at least one molecular species of interest contained in a cleared sample;

(d) pre-focusing the at least one molecular species of interest in the cleared sample, wherein steps (a) through (d) are performed for a number of times sufficient to bring the concentration of the at least one molecular species of interest in the sample up to the level of detection;

(e) electrophoretically separating the molecular species of interest in the cleared sample; and

(f) detecting the separated molecular species of interest.

4. The method of Claim 3 wherein step (c) further comprises removing insoluble contaminants.

5. The method of Claim 3 wherein the molecular species of interest is selected from the group consisting of antibodies, proteins, peptides, peptidomemetics, peptide-nucleic acids, oligonucleotides, aptamers, lipids, polysaccharides, liposaccharides, lipoproteins, glycoproteins and small molecules.

6. A method of detecting at least one molecular species of interest, comprising:

(a) acquiring a crude sample containing at least one molecular species of interest, one or more rough components that are larger

than the molecular species of interest, and one or more fine components that are smaller than the molecular species of interest;

(b) separating from the molecular species of interest at least a portion of a rough component and, optionally, at least a portion of the fine component of the crude sample, thereby producing a cleared sample comprising the at least one molecular species of interest;

(c) introducing the cleared sample into a separation device, said device comprising:

(i) a capillary, at least partially filled with a high-electrolyte buffer, said capillary having an inlet end and an outlet end;

(ii) a means for applying voltage between the inlet end and the outlet end of said capillary; and

(iii) a means for applying pressure differential between the inlet end and the outlet end of said capillary;

(d) pre-focusing the at least one molecular species of interest in the cleared sample, wherein steps (a) through (d) are performed for a number of times sufficient to bring the concentration of the at least one molecular species of interest in the sample up to the level of detection;

(e) electrophoretically separating the molecular species of interest in the cleared sample; and

(f) detecting the separated molecular species of interest.

7. The method of Claim 6 wherein step (b) comprises filtering or centrifuging the sample.

8. The method of Claim 6 wherein step (b) further comprises adjusting acidity of the sample to between about 5 and about 7 pH units.

9. The method of Claim 8 wherein acidity of the sample is adjusted to about 5.5 to about 6.5 pH units.

10. The method of Claim 6 wherein step (b) optionally includes at least partially denaturing the at least one molecular species of interest in the cleared sample.

11. The method of Claim 10 wherein denaturing comprises adding to the cleared sample at least one denaturing agent selected from the group consisting of urea, guanidine hydrochloride, sodium dodecyl sulfate, potassium lauryl sulfate, dithiothreitol, dithioerythritol, trichloroacetic acid, sodium hydroxide, ethylenediamine tetraaceticacid and combinations thereof.

12. The method of Claim 10 wherein denaturing comprises adding to the cleared sample at least one detergent and heating the sample in the presence of said detergent.

13. The method of Claim 12 wherein the sample is heated for a period of time from about 1 minutes to about 10 minutes at a temperature from about 60 °C to about 100 °C.

14. The method of Claim 13 wherein the sample is heated for about 2 minutes at about 90 °C.

15. The method of Claim 12 wherein at least one detergent is sodium dodecyl sulfate.

16. The method of Claim 10 wherein step of separating from the molecular species of interest at least a portion of a rough component and, optionally, at least a portion of the fine component of the crude sample further comprises filtering or centrifuging the cleared sample.

17. The method of Claim 6 wherein step (c) includes introducing a low-electrolyte plug into said separation device, said low-electrolyte plug disposed between the high-electrolyte buffer and the sample at the inlet end of the capillary, thereby forming a boundary between the low-electrolyte plug and the high-electrolyte buffer.

18. The method of Claim 17 wherein said low-electrolyte plug has an acidity that is substantially different from that of the high-electrolyte buffer.

19. The method of Claim 17 wherein the acidity of the high-electrolyte buffer is between about 7 and about 9 pH units.

20. The method of Claim 17 wherein the acidity of the high-electrolyte buffer is about 7.5 to about 8.5 pH units.

21. The method of Claim 17 wherein the low-electrolyte buffer consists essentially of water.

22. The method of Claim 17 wherein step (d) comprises:

(i) applying voltage between the inlet end and the outlet end of the capillary for a period of time sufficient to cause electrophoretic migration of at least one molecular species in the cleared sample up to the boundary between the low-electrolyte plug and the high-electrolyte buffer, thereby pre-focusing and, optionally, desalting the cleared sample; and

(ii) applying a negative pressure differential between the inlet end and the outlet end of the capillary for a period of time sufficient to cause the pre-focused molecular species of interest to be pushed substantially toward the inlet end.

23. The method of Claim 6 wherein step (e) is performed using at least one of the methods selected from the group consisting of capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), capillary isoelectric focusing (CIEF) and capillary isotachophoresis (CITP).

24. The method of Claim 23 wherein separating the molecular species of interest comprises applying voltage between the inlet end and the outlet end of the capillary for a period of time sufficient to separate the molecular species of interest in the sample.

25. A method of detecting at least one molecular species of interest in a sample, comprising:

(a) acquiring a crude sample containing at least one molecular species of interest, at least one rough component, larger than the molecular species of interest, and at least one fine component smaller than the molecular species of interest;

(b) separating the rough component and, optionally, the fine component from the molecular species of interest, thereby producing a cleared sample, thereby producing a cleared sample comprising the at least one molecular species of interest;

(c) optionally, adjusting the acidity of the cleared sample;

(d) optionally, at least partially denaturing the at least one molecular species of interest in the cleared sample;

(e) optionally, removing insoluble contaminants from the cleared sample;

(f) introducing the cleared sample into a separation device, said device comprising:

(i) a capillary, at least partially filled with a high-electrolyte buffer, said capillary having an inlet end and an outlet end;

(ii) a means for applying voltage between the inlet end and the outlet end of said capillary; and

(iii) a means for applying pressure differential between the inlet end and the outlet end of said capillary;

(g) pre-focusing the at least one molecular species of interest in the cleared sample, wherein steps (a) through (g) are performed for a number of times sufficient to bring the concentration of the at least one molecular species of interest in the sample up to the level of detection;

(h) electrophoretically separating the molecular species of interest in the cleared sample; and

(i) detecting the separated molecular species of interest.

26. The method of Claim 25 wherein the molecular species of interest in the sample are selected from the group consisting of antibodies, proteins, peptides, peptidomemetics, peptide-nucleic acids, oligonucleotides, aptamers, lipids, polysaccharides, liposaccharides, lipoproteins, glycoproteins and small molecules.

27. The method of Claim 25 wherein the acidity of the cleared sample is adjusted to a range from about 5.0 to about 7.0 pH units.

28. The method of Claim 25 wherein the optional denaturing step (d) comprises:

(i) adding at least one detergent to the cleared sample; and

(ii) heating the cleared sample in presence of at least one detergent for a period of time from about 1 minute to about 10 minutes to the temperature from about 60 °C to about 100 °C.

29. The method of Claim 28 wherein heating is performed for about 2 minutes at about 90 °C.

30. The method of Claim 28 wherein the detergent is sodium dodecyl sulfate.

31. The method of Claim 25 wherein step (f) includes introducing a low-electrolyte plug into said separation device, said low-electrolyte plug disposed between the high-electrolyte buffer and the cleared sample at the inlet end the capillary, thereby forming a boundary between the low-electrolyte plug and the high-electrolyte buffer.

32. The method of Claim 31 wherein the high-electrolyte buffer and the low-electrolyte plug have substantially different acidities.

33. The method of Claim 31 wherein the high electrolyte buffer has an acidity of about 7.0 to about 9.0 pH units and the low-electrolyte plug has an acidity of about 7.0 pH units.

34. The method of Claim 25 wherein step (g) comprises:

(i) applying voltage between the inlet end and the outlet end of the capillary for a period of time sufficient to cause electrophoretic migration of molecular species of interest in the cleared sample up to the boundary between the low-electrolyte plug and the

high-electrolyte buffer, thereby pre-focusing the cleared sample; and

(ii) applying a pressure differential between the inlet end and the outlet end of the capillary for a period of time sufficient to cause the pre-focused molecular species of interest to be pushed substantially toward the inlet end, thereby concentrating and, optionally, desalting the sample.

35. A method of detecting at least one protein species of interest in a crude sample, comprising:

(a) acquiring a crude sample containing at least one protein species of interest, at least one rough component, larger than the at least one protein species of interest, and at least one fine component smaller than the at least one protein species of interest;

(b) separating the rough component and, optionally, the fine component from the protein species of interest, thereby producing a cleared sample comprising at least one protein species of interest;

(c) optionally, adjusting the acidity of the cleared sample to between about 5.0 and about 7.0 pH units;

(d) optionally, at least partially denaturing the at least one protein species in the cleared sample by

(i) adding at least one detergent to the cleared s ample; and

(ii) heating the cleared sample in the presence of at least one detergent;

(e) optionally, removing insoluble contaminants contained in the cleared sample;

(f) introducing the cleared sample into a separation device comprising:

(i) a capillary, at least partially filled with a high-electrolyte buffer, having an acidity between about 7.0 and about 9.0 pH units, said capillary having an inlet end and an outlet end;

(ii) a means for applying voltage between the inlet end and the outlet end of said capillary; and

(iii) a means for applying pressure differential between the inlet end and the outlet end of said capillary;

whereby a low-electrolyte aqueous solution plug having the acidity of about 7.0 pH units is introduced into the separation device, said low-electrolyte aqueous plug disposed between the high-electrolyte buffer and the cleared sample at the inlet end the capillary, thereby forming a boundary between the low-electrolyte aqueous plug and the high-electrolyte buffer;

(g) pre-focusing and, optionally, desalting the cleared sample, said pre-focusing and desalting comprising:

(i) applying voltage between the inlet end and the outlet end of the capillary for a period of time sufficient to cause electrophoretic migration of the at least one protein species in the cleared sample up to the boundary between the low-electrolyte aqueous plug and the high-electrolyte buffer, thereby pre-focusing and desalting the cleared sample; and

(ii) applying a pressure differential between the inlet end and the outlet end of the capillary for a period of time sufficient to cause the pre-focused protein

species of interest to be pushed substantially toward the inlet end of the capillary;

(h) electrophoretically separating the protein species in the cleared sample, comprising the step of applying voltage between the inlet end and the outlet end of the capillary; and

(i) detecting the separated protein species.

36. The method of Claim 35 wherein optional step (d) comprises:

(i) adding sodium dodecyl sulfate to the cleared sample; and

(ii) heating the cleared sample in the presence of sodium dodecyl sulfate for about 2 minutes at about 90 °C.

37. The method of Claim 35 wherein acidity of the high-electrolyte buffer is about 7.0 to about 9.0 pH units.